II. REMARKS

Claims 1-9 and 11-15 are pending in the subject application. By this Preliminary Amendment, claims 1 and 15 have been amended. Support for the amendment to claim 1 is found in the application papers on page 3, line 15. Claim 15 has been amended to remove an improper dependency as requested by the Office. Accordingly, these amendments do not raise an issue of new matter and entry thereof is respectfully requested. Amended claims 1-9 and 11-15 are presently under examination.

In view of preceding amendments and the remarks which follow, reconsideration and withdrawal of the rejections and objections is respectfully requested.

Prior to addressing the substantive issues, Applicants' undersigned attorney would like to thank the Examiner for the courtesy provided to her on behalf of the Applicants during the July 8, 2003 telephonic interview.

35 U.S.C. § 112, Second Paragraph

The Office indicated that the last reply overcame all grounds for rejection under 35 U.S.C. § 112, second paragraph, but for the rejection of claim 15 because it improperly depends from two claims. In response, claim 15 has been amended in a sincere effort to remove the grounds for rejection. In view of this amendment, reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph is respectfully requested.

35 U.S.C. § 102(b)/§ 103

Claims 11-13 remain rejected under 35 U.S.C. § 102(b) as allegedly anticipated by McAuliffe et al., of record, published February 1999 (vol. 2).

Claims 11 to 15 remain rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Ryan et al. (WO 96/32482, previously cited).

The Office also maintained the rejection of claims 1-9 and 11-15 under 35 U.S.C. § 103(a) as allegedly unpatentable over Ryan et al. (WO 96/32482), in view of DE 2616390 (English abstract provided).

Specifically, the Office remarked that:

> "Applicants state that their product is formulated 'for use' in foodstuffs, yet this is not specifically reflected in the claims. Simple recitation of an intended use of a product does not necessarily change the claimed product, and must result in a specific difference within said product.
>
> Applicant has provided no specific reason regarding the components of the preparation of McAuliffe, as to why this would not be considered a "food grade" preparation. Further, at such point, applicants' arguments would not be commensurate in scope with the claims, as the claims do not recite any particular components that would distinguish the invention over that of the reference.
>
> It is noted that claim 12 recites no additional components other than lacticin 3147, regardless of form.
>
> Applicants state that the product of Ryan et al. is not a "food-grade composition". This flies in the face of the teachings of the reference, as stated previously on the record, regarding the specific use of the freeze-dried lacticin 3147 powder preparation in foods.
>
> Applicants' arguments regarding the potential thermo-instability of the lacticin 3147 protein are not deemed persuasive for the reasons of record. The Office has provided legitimate resources, and specific facts and data, supporting the fact that lacticin 3147 was known to be heat stabile, and was not in any way suspected to be heat labile, as applicants suggest.

> Applicants' statements and presumption at page 10 of the response fail to find support. Applicants state that 'the temperatures required to spray dry the fermentate of the present invention includes an inlet temperature of about 190 degrees C, and an outlet temperature of about 90 degrees C.' However, this is (a) not claimed, and (b) not necessary to one of ordinary skill in the art to arrive at the claimed invention. Reference to DE 2616390 as cited in the rejection under 35 USC 103, specifically shows that the product was spray-dried at 87-97 degrees C, as stated previously on the record. Thus, spray drying at such elevated temperatures as applicant suggests, is unnecessary. Simply because applicant chose to utilize a higher temperature (again, not claimed), does not alter or make patentable the claimed invention."

Applicants respectfully traverse. The claims have been amended consistent with the July 8, 2003 Examiner interview, *i.e.*, by amendment of claim 1 to recite "dairy based medium". This amendment is intended to distinguish the product and process of the invention from that of McAuliffe et al. which Applicants maintain does not enable a method or system for the preparation of a food-grade concentrate comprising lacticin 3147. While McAuliffe et al. recognized that purified lacticin 3147 could in the future be used as a food additive (*see, e.g.*, first and last paragraph of the article) Applicants maintain that the reference does not enable how to make a food-grade product for the reason of record.

Additionally, Applicants do not claim purified lacticin 3147. Applicants claim a process and product produced by a freeze-dried method which does not require a "purification" step. (*See* page 440 of McAuliffe et al. under the heading "Preparation of lacticin 3147" which shows that the product of McAuliffe is the result of several separations and thus, purification steps.)

Ryan et al. also does not teach or suggest the invention because the authors do not disclose how to make nor have they made a food-grade composition. Ryan et al. also discloses a purified lacticin composition. Applicants' invention, in contrast, is not purified lacticin, it is a composition enriched with lacticin, and therefore contains

a mix of proteins and peptides, some of which are lacticin. Moreover, the product claimed by the present application is not lacticin 3147, it is a spray-dried concentrate comprising lacticin 3147 which is quite distinct from the product disclosed in Ryan et al. or McAuliffe et al.

In other words, the *spray-dried* powder of the present invention was developed as a food-grade ingredient and it is the result of a food-grade fermentation process. Again, the *spray-dried* powder of the present invention has **not** been purified at all and will contain many other food grade fermentation products. In contrast in Ryan et al. the *freeze-dried* lacticin described is the result of a purification protocol in which lacticin has been isolated from all other contaminating peptides and proteins. Moreover the purification is achieved using non-food grade chemicals (which include acetone) and as such the resulting lacticin 3147 would not be suitable for consumption. Thus, the reference fails to anticipate because it does not enable a method to prepare a spray-dried food-grade concentrate. The final composition also is different in that Ryan et al. discloses an isolated lacticin where as Applicants' composition has not been purified prior to processing.

With regard to the Examiner's comments concerning the temperature and conditions used to concentrate the composition, the Applicants submit that the temperature and conditions used would not be considered to be obvious to a person skilled in the art. The generation of a spray dried lacticin 3147 powder is not a trivial task. High temperatures are known to denature proteins and the process of freeze-drying will affect proteins differently that spray-drying which require high temperatures. (*See* pages 269 and 270 of Exhibit A, attached hereto). Prior to developing the spray dried product much emphasis was placed on the optimization of lacticin 3147 production through pH controlled fermentations. Considerable experimentation was required prior to selecting the parameters outlined in the present application. The selection of inlet and outlet temperatures for spray-drying are of

particular relevance, since heat denaturation of the lacticin peptides could occur if exposed to thermal processing for any extended period of time. (*See* pages 261 to 265 of Exhibit A, attached hereto). It would not be obvious from the cited art that lacticin 3147 would retain activity following exposure to such high temperatures and conditions. Applicants point out that even during their experimental work they were surprised to find that lacticin 3147 retained full activity after spray drying.

In addition the selection of a growth substrate suitable for spray drying (particularly following pH adjustment during fermentation) is not something immediately obvious.

With regard to the Examiner's comment that it was known that lacticin 3147 was heat stabile, it would be appreciated by those skilled in the art that the bacteriocin would only be heat stabile at certain temperatures. Considerable experimentation would be required in order to determine those temperatures and conditions. Ryan et al. (*see* page 17 and Figure 6) demonstrates the thermostability of lacticin 3147 at different temperatures and pH values. The results in Ryan et al. demonstrate that conditions such as temperature and pH can have an adverse effect on the activity of lacticin 3147.

Applicants maintain that the result of Ryan et al. (see page 17 and Figure 6) demonstrated that lacticin 3147 lost 50% of activity at 121°C. Therefore, Ryan et al. would actually teach away from using inlet and outlet temperatures described in the present application in order to maintain activity. It is submitted therefore that the fact that lacticin 3147 of the present application survived exposure to inlet and outlet temperatures of 190°C and 90°C respectively (*see* page 7, line 27 of Applicants' specification) and retained full activity was a surprising and unexpected finding. Applicants actually expected to see a loss in activity during the production of the powder. Thus the spray dried bacteriocin of the present application is not obvious over Ryan et al.

Furthermore, the work of Ryan et al. was carried out using synthetic media whereas the spray dried powder of the present application was produced using a dairy based substrate. As previously stated, the spray-dried powder of the invention was developed as a food ingredient and it is the result of a food grade fermentation process.

In view of the preceding amendments and remarks, reconsideration and withdrawal of the rejections of the claims under 35 U.S.C. § 102(b) and § 103, are respectfully requested.

III. CONCLUSION

In the unlikely event that the transmittal letter is separated from this document and/or the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 50-2518**, referencing attorney docket no. 2024005-7005492001. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

If a telephone interview would advance examination of the subject application, the Examiner is invited to telephone Antoinette Konski at (650) 849-4950.

Please forward all correspondence concerning the subject application to the undersigned at the address below.

Respectfully submitted,

Date: Oct. 22, 2003

By: Antoinette F. Konski
Reg. No. 34,202

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Attachment: Exhibit A: Pages 261-265, 269 and 270 from Principles of Food Science, Part I, FOOD CHEMISTRY, Fennema, O.W., ed., Marcel Dekker, Inc., New York and Basel (1976).

PRINCIPLES OF FOOD SCIENCE

edited by Owen R. Fennema

PART I

FOOD CHEMISTRY

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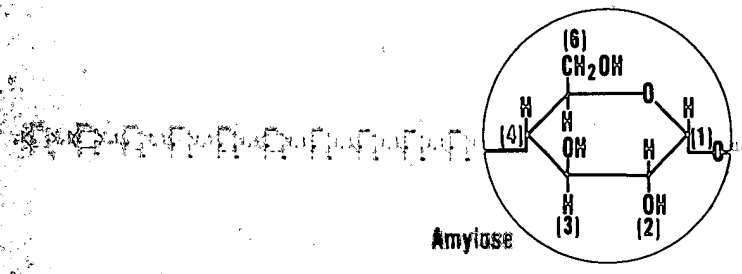
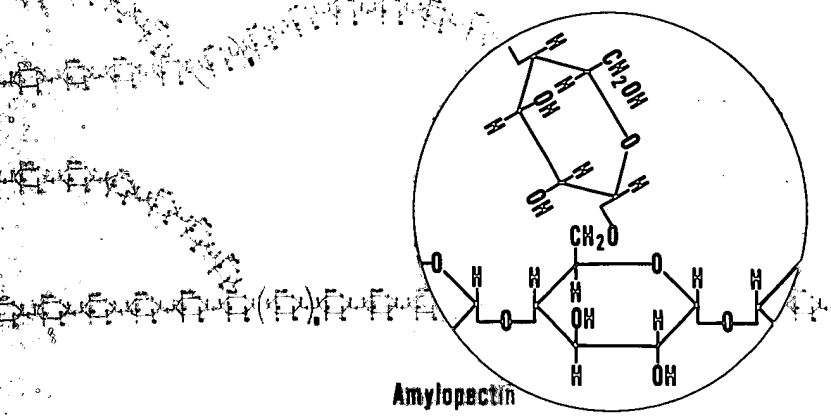


Amylose



Amylopectin

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PART I

FOOD CHEMISTRY

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Department of Food Science
University of Wisconsin-Madison
Madison, Wisconsin

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Ins

a. Flesh Foods. At temperatures above 80°C, sarcoplasmic and myofibrillar proteins coagulate. Oxidation of the sulfhydryl groups of actomyosin leads to formation of disulfide bonds. Hydrogen sulfide is split from actomyosin at about 90°C. Also, at this temperature Maillard reactions between reducing sugars and amino groups of proteins occur rapidly (see Chapter 3). These reactions decrease the nutritional availability of certain amino acids (e.g., lysine). During sterilization most of the collagen is converted to gelatin. Some essential amino acids are damaged during sterilization of meats, especially cysteine, methionine, and lysine [63]. However, this effect is minor in a well-conducted sterilization process.

b. Milk. Complicated changes occur in milk during heat sterilization. Temperatures exceeding that for pasteurization (72°C) cause instability and coagulation of proteins. Depending on the time-temperature relationships, heat treatments may partially dephosphorylate casein, denature whey proteins, initiate protein-protein and protein-lactose interactions, etc. Several articles [79,111,132] cover these complex changes in detail.

3. SPECIAL PROBLEMS IN HEAT PROCESSING

Of all the major food-processing treatments in current use, heat processing has the most important effect on protein quality. Depending on such factors as time, temperature, moisture content, and the presence or absence of reducing substances, heat treatments may have either a beneficial or detrimental influence.

a. Beneficial Effects. Most proteins of plant origin are nutritionally improved by heating. Trypsin inhibitors and other antinutritional components of several seed proteins are inactivated or destroyed by proper heat treatment. Wheat, rye, and to a lesser extent rice and oats contain a trypsin inhibitor that is destroyed by cooking. Digestibility and availability of the sulfur amino acids also are improved by conventional heat treatments. This is particularly true in soybeans and peanuts. Furthermore, the availabilities of methionine, tryptophan, and threonine are greater in bread than in unground wheat [9]. However, heat treatments must be applied with care so that undesirable effects are minimized.

b. Detrimental Effects. Excessive heating can result in a reduction of palatability and a substantial decrease in nutritive value. Three different types of reactions can result in decreased nutritive value of proteins during improper heat processing: oxidation of amino acids, alteration of some of the linkages between amino acids so that their release during digestion is delayed, and formation of new amino acid linkages that are not subject to hydrolysis by digestive enzymes.

The charring that accompanies gross overheating results in destruction of amino acids and losses of nitrogen, sulfur, and dry weight. Although the effects of pyrolysis complicate the reactions producing these irreversible changes, deamination, decarboxylation, and the oxidation of sulfur are known to occur.

Delayed release of amino acids during digestion may disrupt protein synthesis in body tissues by preventing the simultaneous availability of all essential amino

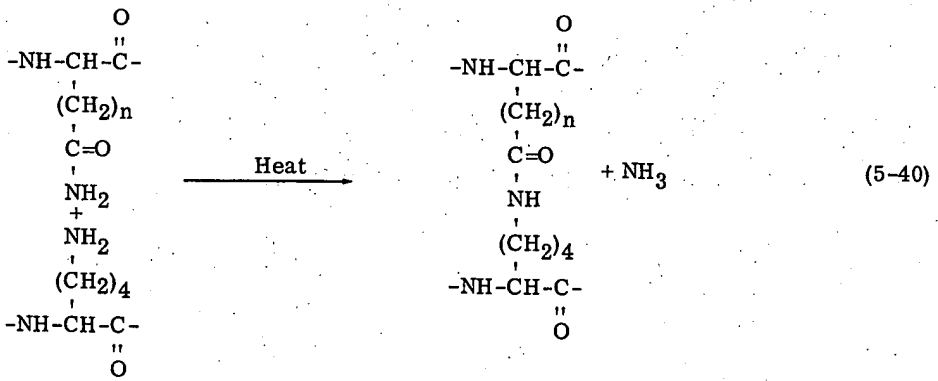
acids. Those that cannot be used in protein synthesis are biologically oxidized [9]. Recent studies have yielded data suggesting that the enzymic release of amino acids can be altered by severely heating proteins [49].

Heat processing can result in the formation of linkages that cannot be hydrolyzed during digestion [9]. Thus, the nutritional availability of some amino acids is markedly reduced even though they sometimes can be recovered by acid hydrolysis. It has been well established that the Maillard or "nonenzymic browning" reaction can account for much of the damaging effect of heat on the protein quality of foods that contain reducing sugars, such as lactose or glucose [83]. Sucrose can also yield reducing sugars if conditions are suitable for its hydrolysis. Lysine readily reacts with lactose to form an amino sugar. The ϵ-amino group of lysine is the preferential site for initiation of the Maillard reaction. The initial Maillard reaction products (prior to the Shiff base) can be cleaved by strong acid but not by enzymic digestion in the gastrointestinal tract [89]. Thus, "bound" lysine has no nutritional value since it cannot be utilized by the body. Arginine, tryptophan, histidine, and threonine also react readily with reducing groups of carbohydrate materials during heating.

The formation of amino sugars may interfere with the specificity requirements for enzyme hydrolysis of proteins in such a manner that amino acids adjacent to the amino sugar site remain unhydrolyzed during digestive processes [43]. Thus, Maillard browning can reduce the availability of many amino acids not directly involved in the reaction.

As the Maillard reaction proceeds, reaction products polymerize (melanoidins), browning intensifies, and significant amounts of amino acids are destroyed (chemically altered irreversibly). In addition to reducing sugars, fats or fatty acids that are undergoing autoxidation may contribute carbonyl groups for a Maillard-type reaction [80].

In the absence of reducing substances, protein-protein interactions (C-N links) can be initiated by exposing proteinaceous tissues to high temperatures (>110°C) for substantial lengths of time (> 24 hr). New bonds or crosslinkages, such as :CH·N: links, may replace peptide bonds (·CO·NH·). Digestive enzymes are clearly unable to cleave :CH·N: linkages and thus the bound amino acids are not absorbed [43]. According to Bjarnason and Carpenter [14], lysine that becomes unavailable during the heating of carbohydrate-free proteins does so because unnatural amide bonds form between the ϵ-amino group of lysine and the amide groups of asparagine and glutamine (see below). The reacting components may reside either in the same peptide chain or in neighboring molecules.



(5-40)

Such crosslinking reduces the nutritional availability of all essential amino acids.

Cystine is the amino acid most sensitive to heat. During heating of carbohydrate-free proteins at 115°C for 27 hr (note severity of heating), 50-60% of the cystine is destroyed and H_2S is evolved. The following reactions [14] are at least partly responsible for destruction of cystine during the heating of proteins in the absence of oxygen:

$$R\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}R + H_2O \longrightarrow R\text{-}CH_2\text{-}SH + R\text{-}CH_2\text{-}SOH \quad (5\text{-}41)$$

$$R\text{-}CH_2\text{-}SOH \longrightarrow R\text{-}CHO + H_2S \quad (5\text{-}42)$$

Cystine loss during heating of proteins becomes of major nutritional significance only if this makes sulfur-bearing amino acids limiting. Generally, the nutritional quality of heated proteins is affected less by loss of cystine than by other changes [14]. Exceptions occur in diets in which sulfur amino acids tend to be limiting. Examples include diets that are heavily dependent on milk, cottonseed protein, or fish meals (limited by methionine plus cystine [9]).

The puffing-exploding type of processing used in the manufacture of certain breakfast cereals causes severe protein damage. Rolled oats boiled in water for 15 min and dried for 15 sec at 130°C or cooked in steam for 2 min at 100-lb pressure, then exploded and dried for 2 min at 200°C have protein efficiency ratios (PER) of 1.6. Conversely, when oats are puffed by heating for 5 min at 122°C and for 2 min at 189°C, then exploded, the PER decreases to 0.3 [92]. The puffing-exploding heat treatment disrupts many chemical bonds and results in the destruction of substantial amounts of essential amino acids.

Toasting of cereal products also results in considerable damage to the nutritive quality of protein, particularly in the outer layers which are subjected to the greatest heat intensity. Much of this damage is due to the loss and/or unavailability of lysine. The original nutritive value of toasted cereals can be completely restored by the addition of lysine [9].

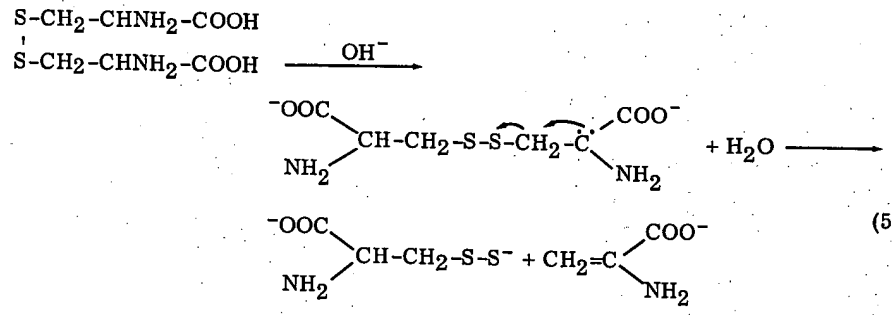
Although some damage or destruction of protein invariably results from normal commercial heat treatments, such losses are considered as relatively insignificant. In contrast, the more harsh heat treatments such as the puffing-exploding and toasting processes used in the manufacture of certain breakfast cereals are significantly destructive to protein. These foods, however, do not contribute much protein to the American diet since they comprise only a small portion of the daily food intake. Furthermore, most of these products are consumed with milk, which compensates for the lysine lost during these specialized heat-processing treatments [8,9].

c. Alkali Treatment. The use of alkali in food processing, especially when used in conjunction with heat, is cause for some concern because of its damaging effects on protein quality. Alkali treatments are becoming more common in the food industry for preparing protein concentrates and isolates, for modifying proteins to obtain or enhance specific functional properties (foaming, emulsifying, etc.), and for producing protein solutions suitable for spinning fibers. Alkali treatment of proteins may result in formation of new amino acids, such as lysinoalanine, lanthionine, and ornithinoalanine. Cystine, lysine, arginine, and possibly serine are the amino acids involved in these modifications. Since sulfur amino acids and

lysine are limiting in a majority of food proteins; reduced nitritive value may result from their exposure to alkali.

Exposure of a high-protein product, soy protein isolate, to aqueous alkali (pH 12.2) for 4 hr at 40°C results in the formation of lysinoalanine (LAL), which is poorly absorbed [38]. The amount of LAL formed increases with rising temperature and with increasing exposure time. The presence of LAL in alkali-treated soy protein isolate is accompanied by a reduction in the cystine and lysine contents. A more severe treatment of the protein with alkali (pH 12.2) at temperatures exceeding 60°C also causes decreases in serine content and in protein digestibility.

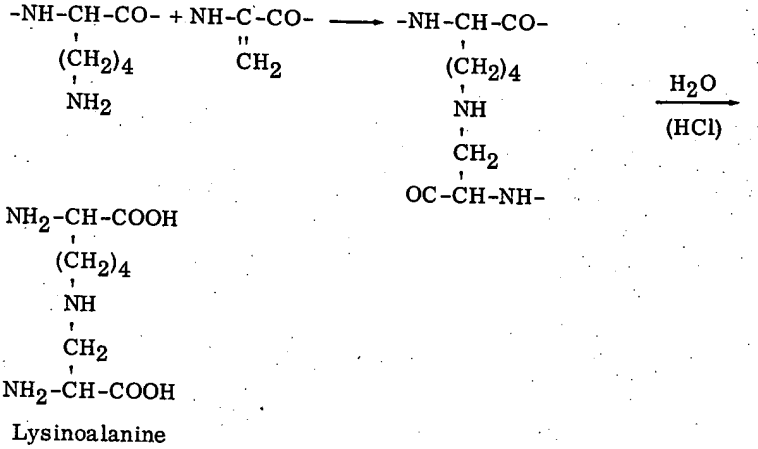
The primary change induced in proteins by alkali treatment is apparently a β-elimination reaction [27] leading to formation of dehydroalanine residues from cystine:



(5-43)

Dehydroalanine

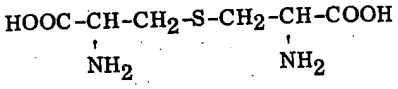
Lysinoalanine is formed by the addition of an ϵ-amino group of a lysyl residue across the double bond of dehydroalanine as indicated below [98]:



(5-44)

Lysinoalanine

During alkaline hydrolysis of proteins (boiling wool or lactalbumin for 1 hr in either 0.1N NaOH or 2% Na_2CO_3), Horn et al. [70] noted that cystine residues lost one of the sulfur atoms and were converted into a thiol ether diamino acid, lanthionine:



The heating of egg white (pH 9.0) for 60 min at temperatures exceeding 60°C results in H_2S production and the formation of lanthionine [52]. It was concluded that H_2S was produced from cysteine as a result of nonenzymic reactions while lanthionine was formed by an unknown reaction mechanism. In an earlier study, Chapeville and Fromageot [29] isolated the enzyme cysteine lyase from egg yolk. This enzyme was found to exert a desulfhydration effect and also could lead to the production of ammonia, pyruvic acid, and lanthionine as shown in Figure 5-17. A similar reaction mechanism may function during the heating of egg white [52].

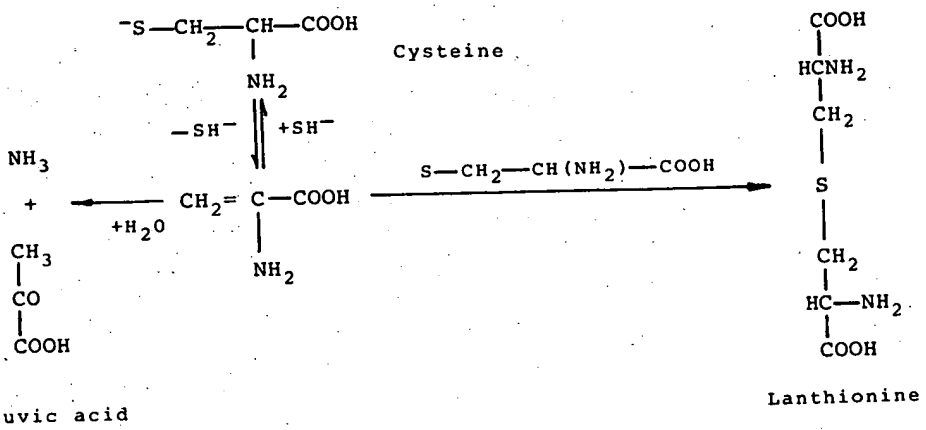
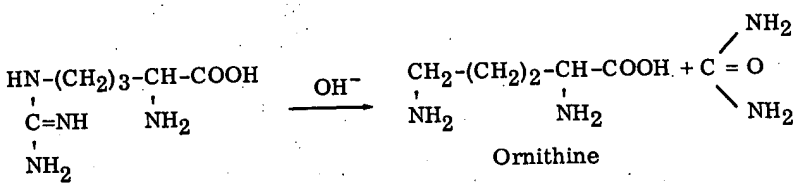


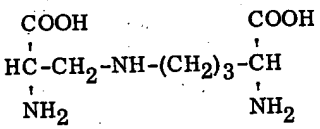
FIG. 5-17. A possible pathway leading to the formation of lanthionine during heating of egg white. Reprinted from Ref. [52], p. 15, courtesy of A. C. Germs.

Free or combined arginine can be degraded by alkali to form ornithine:



(5-45)

The δ-amino groups of combined ornithine can react with dehydroalanine (produced as decomposition products of cystine and/or serine residues) to yield ornithinoalanine [149]:



2. VACUUM DRYING

A moderate vacuum of 5-10 mm Hg absolute pressure is used in this procedure. Flesh foods dried by this method are damaged less than by conventional dehydration. The absence of air retards oxidative changes, and the low drying temperatures decrease nonenzymic browning and other chemical changes [110].

3. FREEZE DRYING

In this process, the product is frozen, the pressure is reduced to 1 mm Hg or less, and water is removed by sublimation. The product retains its original size and shape although it shows considerable porosity when dried. This porosity enhances reconstitution [110].

Freeze drying is the best process for dehydrating flesh foods. However, even under optimum operating conditions there are indications that the proteins of freeze dried meat are apparently altered. For example, freeze-dried meat after rehydration and cooking is less tender and juicy than cooked meat that has not been freeze dried. The decreased WHC and toughness may result when actomyosin molecules come closer together, as water is removed during dehydration. Some unfolding of peptide chains may also occur during this period. This is believed to result in aggregation of myofibrillar proteins as new intermolecular salt and/or metal bonds are formed [63,69].

The extent of damage in freeze dried meat or fish is always greater than that resulting merely from a freeze-thaw process. Denaturation of myofibrillar proteins during freeze drying is caused by the removal of "bound" water rather than by the freezing process [34,63].

Dehydration also can result in other deteriorative changes. These involve a loss of nutritive value, especially destruction of lysine by nonenzymic browning, and decreased digestibility which results from interactions of denatured proteins with lipids and carbohydrates. The amount of damage occuring during dehydration of flesh foods increases with the severity of the operating conditions. Elevated temperatures and long exposure to air are particularly harmful. In most instances, however, freeze-dried flesh foods are similar to the fresh products with respect to content of essential amino acids and digestibility.

4. SPRAY DRYING

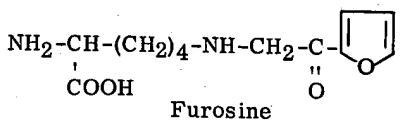
Spray drying is generally used to produce dehydrated eggs and milk products. In spray drying, the liquid is finely atomized into a stream of rapidly moving hot air. Although heat is the principal agent causing protein alteration during spray drying, surface forces cannot be ignored [32]. In the spray drying of liquid egg white, for example, the shear forces encountered during pumping and atomization operations may have a harmful effect on the protein and on the functional properties of the product. The great surface area generated by atomization also may cause surface denaturation of proteins and a subsequent reduction in whipping and other functional properties. However, the percentage of protein that is usually denatured under standard atomization conditions is relatively small [10].

During spray drying of milk and milk products under properly controlled conditions, changes in milk protein structures and solubilities are relatively minor [61].

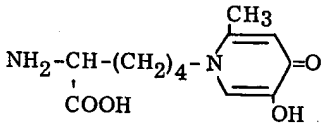
5. DRUM DRYING

In drum or "roller" drying, the product is dried in a thin film on a rotating drum that is internally heated with steam. Many operational variables related to time and temperature must be controlled rather rigidly to produce high-quality products [61]. Occasionally, the dry product has a scorched flavor and reduced protein solubility since severe time-temperature conditions are not uncommon.

Two new amino acids, furosine and pyridosine, have been identified in scorched drum-dried milk powders [47,48]. They arise from lysine and their structures are given below:



Furosine



Pyridosine

Furosine and pyridosine are found in relatively large amounts in acid hydrolyzates of drum-dried milk powders, in trace amounts in hydrolyzates of spray-dried milk products, and not at all in freeze-dried milk. Monitoring the formation of furosine might be a useful indicator of heat damage to proteins since it forms well before nutritive damage can be detected. Neither of these new amino acids is readily absorbed in the intestine.

D. Effect of Radiation on Proteins

Although the processing of foods with ionizing radiation has not yet been approved by the Food and Drug Administration of the United States, there is still considerable interest in this prospective means of food preservation. Radiation damage to proteins in aqueous systems, such as foods, is attributable mainly to indirect effects, i.e., irradiation of water produces free radicals (·OH, H·, etc.) and hydrated free electrons (e^-_{aq}), and these in turn interact with and alter proteins [117]. Since foods contain many components capable of reacting with these water radicals, only a small proportion of these radicals may react with proteins.

Despite considerable research, knowledge of the radiation chemistry of proteins in aqueous solution is still rather limited. More is known about the radiation chemistry of amino acids and simple peptides and it is assumed that the major radiolytic reactions of proteins parallel those of amino acids and peptides [64]. Thus, generalizations drawn from the radiation chemistry of amino acids and peptides appear useful.